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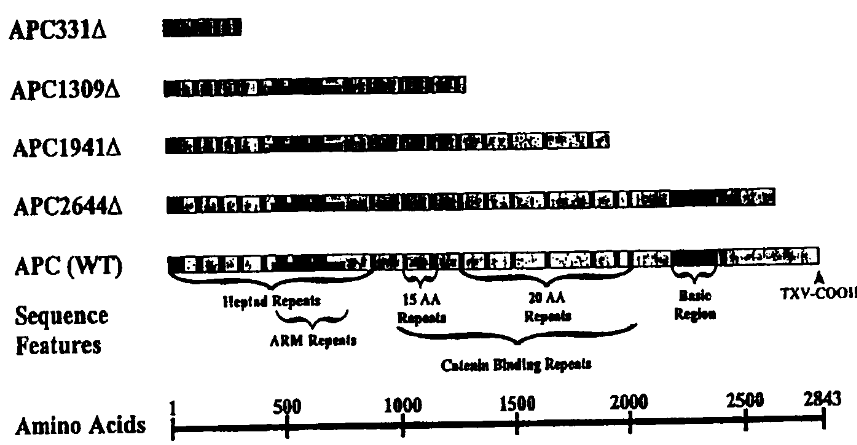

(57) Abstract

The APC tumor suppressor protein binds to β-catenin, a protein recently shown to interact with Tcf/Lef transcription factors. Here, the gene encoding a Tcf family member that is expressed in colonic epithelium (*hTcf-4*) was cloned and characterized. hTcf-4 transactivates transcription only when associated with β-catenin. Nuclei of APC$^{-/-}$ colon carcinoma cells were found to contain a stable β-catenin-hTCF-4 complex that was constitutively active, as measured by transcription of a Tcf reporter gene. Reintroduction of APC removed β-catenin from hTcf4 and abrogated the transcriptional transactivation. Constitutive transcription of TCF target genes, caused by loss of APC function, may be a crucial event in the early transformation of colonic epithelium. It is also shown here that the products of mutant *APC* genes found in colorectal tumors are defective in regulating β-catenin/Tcf-4 transcriptional activation. Furthermore, colorectal tumors with intact *APC* genes were shown to contain subtle activating mutations of β-catenin that altered functionally significant phosphorylation sites. These results indicate that regulation of β-catenin is critical to APC's tumor suppressive effect and that this regulation can be circumvented by mutations in either APC or β-catenin.

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β-CATENIN, TCF-4, AND APC INTERACT TO PREVENT CANCER

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TECHNICAL FIELD OF THE INVENTION

This invention is related to the field of cancer diagnostics and therapeutics. More particularly it relates to methods for diagnosing and treating cancers associated with APC or β-catenin mutations.

BACKGROUND OF THE INVENTION

Mutations of the adenomatous polyposis coli (*APC*) gene are the most common disease-causing genetic events in humans; approximately 50% of the population will develop colorectal polyps initiated by such mutations during a normal life span (*14*). Individuals who inherit *APC* mutations develop thousands of colorectal tumors, consistent with APC's tumor suppressor or "gatekeeping" role in colorectal tumorigenesis (*15,16*). APC homodimerizes through its amino-terminus (*17*), and interacts with at least six other proteins: β-catenin (18), γ-catenin (plakoglobin) (*19*), tubulin (*20*), EB1 (*21*), hDLG, a homologue of a *Drosophila* tumor suppressor protein (*22*), and ZW3/GSK3β kinase (*23*). Whether any of these interacting proteins communicate APC growth-controlling signals is unknown. Thus

there is a need in the art for a fuller understanding of how the tumor suppressor gene APC functions in cells.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide human nucleotide sequences encoding transcriptional activation proteins.

It is another object of the present invention to provide isolated preparations of transcriptional activation proteins.

It is an object of the present invention to provide methods of determining the presence or absence in a cell of wild-type APC or a downstream protein in the APC transcription regulatory pathway.

Another object of the invention is to provide methods of identifying candidate drugs for use in Familial Adenomatous Polyposis (FAP) patients or patients with increased risk of developing cancer.

It is yet another object of the invention to provide methods of identifying candidate drugs for the treatment of cancer patients, in particular those with *APC* or β-catenin mutations.

Another object of the invention is to provide a method for diagnosing cancer in a sample suspected of being neoplastic.

Another object of the invention is to provide a method for treating a patient with colorectal cancer or other cancer associated with FAP.

These and other objects of the invention are achieved by providing one or more of the embodiments described below. In one embodiment of the invention an intron-free DNA molecule is provided which encodes Tcf-4 protein as shown in SEQ ID NO: 2 or 4.

According to another embodiment of the invention an isolated Tcf-4 protein is provided. The protein is substantially free of other human proteins, and has a sequence as shown in SEQ ID NO: 2 or 4.

In another embodiment of the invention a method is provided for determining the presence or absence in a cell of wild-type APC or a

downstream protein in the APC transcription regulatory pathway. The method comprises the steps of:

introducing a Tcf-responsive reporter gene into the cell; and

measuring transcription of said reporter gene; wherein a cell which supports active transcription of said reporter gene does not have wild-type APC or does not have a wild-type downstream protein in the APC transcription regulatory pathway.

According to yet another embodiment of the invention a method is provided for determining the presence or absence in a cell of wild-type APC. The method comprises the steps of:

contacting a Tcf-responsive reporter gene with a lysate of the cell; and

measuring transcription of said reporter gene; wherein a lysate which inhibits said transcription has wild-type APC.

In still another embodiment of the invention a method of identifying candidate drugs is provided. The drugs may be useful for treatment of FAP or other cancer patients or patients with increased risk of developing cancer. The method comprises the steps of:

contacting a cell having no wild-type APC or a mutant β-catenin with a test compound;

measuring transcription of a Tcf-responsive reporter gene, wherein a test compound which inhibits the transcription of the reporter gene is a candidate drug for cancer therapy.

According to yet another aspect of the invention another method is provided for identifying candidate drugs for use in for use in FAP patients, colon cancer patients, patients with mutations in β-catenin or *APC*, or patients with increased risk of developing cancer. The method, comprises the steps of:

contacting a Tcf-responsive reporter gene with a test compound under conditions in which the reporter gene is transcribed in the absence of the test compound; and

measuring transcription of the Tcf-responsive reporter gene; wherein a test compound which inhibits said transcription is a candidate drug for cancer therapy.

According to another aspect of the invention a method is provided for identifying candidate drugs for use in FAP patients or patients with increased risk of developing cancer. The method comprises the steps of:

contacting a test compound with β-catenin and Tcf-4 under conditions in which β-catenin and Tcf-4 bind to each other; and

determining whether the test compound inhibits the binding of β-catenin and Tcf-4, a test compound which inhibits the binding being a candidate for cancer therapy or prophylaxis.

According to still another embodiment of the invention a method is provided for diagnosing cancer in a sample suspected of being neoplastic. the method comprises the steps of:

comparing a CTNNB sequence found in the sample to a second CTNNB sequence found in a normal tissue, wherein a difference between the first and second sequence is an indicator of cancer.

According to another aspect of the invention a method is provided for treating a patient with colorectal cancer or other cancer associated with FAP. The method comprises the step of:

administering to the patient a nucleotide sequence comprising a portion of the APC coding sequence, said portion consisting of the β-catenin binding site.

According to another aspect of the invention a method is provided for treating a patient with colorectal cancer or other cancer associated with FAP. The method comprises the step of:

administering to the patient a polypeptide comprising a portion of the APC coding sequence, said portion consisting of the β-catenin binding site.

The present invention thus provides the art with diagnostic, therapeutic and drug discovery methods especially useful for FAP and other cancers with *APC* or β-catenin mutations.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1. Sequence comparison of hTcf-4 and hTcf-1.

Two alternative splice forms of *hTcf-4* were identified, each encoding a different COOH-terminus. One form (hTcf-4E) was homologous to hTCF-IE (top) (7); the other form (hTcf-4B) was homologous to hTcf-1B (bottom). The highly conserved NH_2-terminal interaction domain and the High Mobility Group (HMG) box DNA-binding region are boxed. Abbreviations for the amino acids are: A. Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; IC, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; P, Ar g; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. The nucleotide sequence has been deposited in GenBank (accession number:)

Fig. 2. Analysis of *hTcf-4* expression in colonic epithelium.

(Fig. 2A) Northern blot analysis of *hTcf-4, hTcf-1, hLef-I* expression in Jurkat T cells (lane 1); colonic mucosa (lane 2); colon carcinoma cell lines DLD-1 (lane 3), HCT116 (lane 4); SW480 (lane 5); SW620 (lane 6); HT29 (lane 7). Lane 2 contains 5 μg total RNA; all others contain 15 μg total RNA. The positions of 18S and 28S ribosomal RNAs are shown. EtBr, ethidium bromide stain. (Fig. 2B) In situ hybridization of healthy human colon tissue to an *hTcf-4* probe. (Fig. 2C) In situ hybridization to a negative control probe (a fragment of the *E. coli* neomycin resistance gene).

Fig. 3. Transactivational properties of β-catenin/hTcf-4.

All reporter assays were performed as duplicate transfections. For each condition, both values are shown. (**Fig.** 3A) Reporter gene assays in IIA1.6 B cells. Cells were transfected by electroporation with 1 μg luciferase reporter plasmid, 5 μg β-catenin expression plasmid, and 3 II-hTcf-4 expression plasmids. Empty pCDNA was added to a total of 10 μg, plasmid DNA. (**Fig.** 3B) Reporter gene assays in SW480 colon carcinoma cells. Cells were transfected with 0.3 μg, of the indicated luciferase reporter gene, 0.7 μg pCATCONTROL as internal control, the indicated amounts of pCMVNeoAPC, and empty PCDNA to a total of 2.5 μg plasmid DNA. Control CAT values are given in the right panel.

Fig. 4. Constitutive presence of β-catenin-hTcf-4 complexes in $APC^{-/-}$ cells. Gel retardation assays were performed on nuclear extracts from the indicated cell lines before and after a 20-hour exposure to Zn^{++}. Samples in lanes 1, 4, 7, 10 were incubated under standard conditions. To the samples in lanes 2, 5, 8, 11, 0.25 μg, anti β-catenin was added. To the samples in lanes 3, 6, 9, 12, 0.25 μg of a control (human CD4) antibody was added. N.S., nonspecific band also observed with mutant (nonbinding) probe (lane Mt).

Fig. 5. Effects of *APC* mutations on CRT. (**Fig. 5A**) Schematics of wild-type (WT) and mutant APC. APC is a 2843-amino-acid (AA) protein (*32*) with contains armadillo (ARM) repeats in the amino-terminus (*33*), 15 and 20 AA β-catenin-binding repeats in the central region (*18,19*), and a basic region in the carboxyl-terminus (*32*). The carboxyl-terminus also contains a TXV sequence which mediates DLG binding (*22*). (**Fig. 5B**) Effects of WT and mutant APC on CRT. SW480 cells containing endogenous mutant *APC* were transfected with the APC expression vectors shown in (**Fig. 5A**) and CRT was measured. Cells were transfected with increasing amounts of WT *APC* (0, 0.15 and 0.5 μg) or 0.5 μg mutant

APC. CRT reporter activities are expressed relative to assays containing no WT APC and are the means of three replicates. Error bars represent standard deviations.

Lipofectamine was used to cotransfect SW480 cells with an internal control (0.5 μg pCMV-βgal), a reporter construct (0.5 μg pTOPFLASH or pFOPFLASH) and the indicated amount of the various APC expression vectors. The pTOPFLASH reporter contained an optimized Tcf-binding site 5' of a luciferase reporter gene, whereas pFOPFLASH contained a mutated site that does not bind Tcf . The amount of DNA in each transfection was kept constant by addition of an appropriate amount of empty expression vector (pCEP4). Luciferase and β-galactosidase activities were determined 16 hours after transfection. Luciferase activity was corrected for transfection efficiency (using the control β-galactosidase activity) and nonspecific transcription (using the pFOPFLASH control).

Fig. 6. Evaluation of CRT in colorectal cancer cell lines with WT APC. **(Fig. 6A)** Immunoblot of endogenous APC in the DLD1, SW480, HCT116, SW48 and 293 cell lines, developed with APC monoclonal antibody FE9 (*34*). **(Fig. 6B)** Effects of exogenous WT APC on CRT in cell lines with endogenous mutated or WT APC. Cells were transfected with increasing amounts (0, 0.15 μg, 0.5 μg for DLD1 and SW48; 0, 0.5 μg, 5 μg for HCT116) of WT APC or APC1309Δ mutant (0.5 μg for DLD1 and SW48; 5 μg for HCT116) and CRT was assessed as in Fig. 5. CRT reporter activities are expressed relative to activity in extracts without exogenous APC and are the means of three replicates. Error bars represent standard deviations.

Fig. 7. Evaluation of β-catenin in colorectal cancer cell lines with WT APC. **(Fig. 7A)** Immunoblot of the cell lines used in this study, developed with β-catenin monoclonal C19220 (Transduction Laboratories, Lexington, KY)(*31*). **(Fig. 7B)** Sequence of *CTNNB1* in HCT116 and SW48 . Overlapping segments constituting the entire *CTNNB1* were amplified by

RT-PCR from SW480, DLD1, HCT116, and SW48 cells, and sequenced directly with ThermoSequenase (Amersham). In the case of HCT116, a PCR product containing the deleted region was also cloned into pCI-neo (Promega, Madison) and multiple clones corresponding to each allele were individually sequenced.

The left panel (nts 121 to 143 from HCT116) reveals the presence of a deletion in addition to the WT sequence. The middle panel (antisense strand 156 to 113 of the WT and deleted alleles of HCT116) reveals the 3-bp deletion (ΔTCT) that removed codon 45 in half the clones. The right panel (nts 80 to 113 from SW48) reveals a C to A transition affecting codon 33 (TCT to TAT). (**Fig. 7C**) Schematic of β-catenin illustrating the armadillo repeats (*33*) and negative regulatory domain. The residues in larger type fit the consensus sequence for GSK3β phosphorylation (*29*) and those in bold have been demonstrated to affect down regulation of β-catenin through GSK3β phosphorylation in Xenopus embryos (*27*). The five mutations found in human colon cancers are indicated at the top.

Fig. 8. Functional evaluation of β-catenin mutants. (**Fig. 8A**) Constitutive nuclear complex of β-catenin and Tcf in HCT116 cells. The presence of nuclear β-catenin-Tcf complexes was assessed by gel shift assays. Lanes 1 to 3, optimal Tcf retardation probe shifted with nuclear extract from HCT116 cells with addition of no antibody (lane 1), anti β-catenin (0.25 μg, lane 2), or an irrelevant antibody (0.25 μg, lane 3). Lane 4, mutant Tcf retardation probe shifted with nuclear extract from HCT116 cells. n.s., nonspecific shifting seen with the mutant probe. (**Fig. 8B**) Effects of the β-catenin mutations on CRT. 293 cells were transfected with WT (WT) or mutant (Δ45, S33Y) β-catenin and CRT was assessed. CRT reporter activities are expressed relative to WT β-catenin and are the means of three replicates. Error bars represent standard deviations. β-catenin expression constructs were prepared as follows. WT *CTNNB1* was

amplified by RT-PCR from SW480 cells and cloned into the mammalian expression vector pCI-neo (Promega) to produce pCI-neo-β-cat. The pCI-neo-β-cat Δ45 and S33Y were generated by replacing codons 1 to 89 in pCI-neo-β-cat with a PCR product encoding the equivalent region from HCT116 or SW48 cDNA, respectively. The structures of all constructs were verified by sequence analysis. Lipofectamine was used to cotransfect 293 cells with an internal control (0.1 μg CMV-βgal), a reporter (0.5 μg pTOPFLASH or pFOPFLASH), a Tcf-4 expression vector (0.5 μg pCDNA-TCF4), and β-catenin (0.5 μg) or dominant negative hTcf-4 1.0 μg) expression vectors. CRT was determined as described above.

DETAILED DESCRIPTION

It is a discovery of the present invention that hTcf-4 binds to β-catenin and activates transcription in colorectal epithelial cells. Moreover, it has now been found that APC regulates this transcriptional activation, at least in part by binding to β-catenin. In colorectal cancer cells this regulation is frequently abrogated, either by mutation of APC or by mutation of β-catenin.

Two alternative splice forms of human Tcf-4 have been found. One form (hTcf-4E) is homologous to hTcf-1E and the other (hTcf-4B) is homologous to hTcf-1B. The sequence of the nucleotide and amino acid sequences are shown in SEQ ID NOs: 1-4. The coding sequences and proteins can be used in assays as described below. Intron-free DNA molecules are provided which are originally made by reverse transcription of a mRNA molecule. They can be propagated in cells or amplified as is desired. Isolated Tcf-4 proteins can be provided substantially free of other human proteins if, for example, the nucleotide sequences are expressed in non-human cells. Methods and vectors for achieving such expression are well known in the art. Choice of such expression means is made by the skilled artisan according to the desired usage and convenience.

Cells can be tested to determine if they have a wild-type APC or a wild-type downstream protein in the APC transcription regulatory pathway, called herein the CRT pathway (β-catenin/Tcf-regulated transcription). One protein within the CRT pathway which has been identified as a target of mutations in human cancers is β-catenin (encoded by the *CTNNB1* gene). Other parts of the pathway are also likely to be targets. Although the target genes of the CRT pathway have not been identified, they can be readily identified using the system disclosed here. Genes which are differentially transcribed in the presence of wild-type and mutant *CTNNB1*, for example, can be identified.

Tcf-responsive reporter genes are those constructs which comprise a readily detectable or assayable gene (such as luciferase, β- galactosidase, chloramphenicol acetyltransferase) linked *in cis* to a Tcf-responsive element. Such responsive elements are known in the art (7) and any such elements can be used. An optimal Tcf motif contains the sequence CCTTTGATC. From one to twenty copies, and preferably from three to six copies, of the motif may be used. Mutation of the sequence to CCTTTGGCC abrogates responsiveness. Another necessary part of such constructs is a minimal promoter, such as the *c-Fos* or the Herpes virus thymidine kinase promoter. Transcription of the reporter gene may be performed by any means known in the art, usually by assaying for the activity of the encoded gene, although immunological detection methods can also be used. In addition, transcription can be monitored by measuring the transcribed mRNA directly, typically using oligonucleotide probes.

As shown below, a cell which has a wild-type APC protein will inhibit CRT. However, most mutations in *APC* render APC unable to inhibit CRT. Similarly, certain mutations in *CTNNB1* render β-catenin super-active and/or refractory to the inhibition by APC. Thus measuring Tcf-responsive reporter gene transcription is an indication of the status of

APC and *CTNNB1*. Mutations in both of these genes are associated with cancers and therefore provides diagnostic and prognostic information.

Assays for CRT can be accomplished *in vitro* or in cells. If the assay is to be accomplished in cells, then a Tcf-responsive reporter gene must be introduced into the cell. Any means for introducing genetic material into cells can be used, including but not limited to infection, transfection, electroporation. If the assay is to be performed *in vitro* then the components for transcription must be present. These include suitable buffers, RNA polymerase, as well as ribonucleotides. If the protein product is to be assayed, then the components for translation must also be present, such as ribosomes, and amino acids.

These assays can also be used to screen compounds for potential as anti-cancer therapeutic agents. Using either the *in vitro* or cell form of the assay, test compounds can be introduced to determine whether they are able to mimic the effect of wild-type APC or to convert a mutant APC into a form which is able to inhibit CRT or a mutant β-catenin into a form which is regulatable by APC. In addition, compounds can be tested for the ability to inhibit the binding of β-catenin and Tcf-4, thus mimicking the action of APC. Such a test can be conducted *in vitro* or *in vivo*, for example using a two hybrid assay.

A means for diagnosis of cancers is the result of the observation that *CTNNB1* mutations are found in tumor cells, especially those which have wild-type APC. Such mutations can be found, *inter alia*, by sequencing either the gene or the protein found in a sample. Functional assays can also be used, such as whether β-catenin binds to APC or Tcf-4, or whether it is capable of mediating CRT. Sequences can be compared to those found in a normal tissue of a human, especially the same human who provided the sample being tested. Suitable tumors for testing include, but are not limited to those which are associated with FAP. Suitable tumors include colorectal cancer, thyroid cancer, brain cancer, medulloblastoma, desmoid tumor,

osteoma, breast cancer, and head and neck cancer. Because APC mutations are so frequent, and because it appears that APC mutations do not occur in the same tumors as CTNNB1 mutations, one can prescreen samples for APC mutations before performing a CTNNB1 determination.

The portion of the APC gene which encodes the β-catenin binding site can be used in a gene therapy format. Suitable techniques are known in the art for administering genes to tumors, and any such technique can be used. Suitable expression vectors are also known in the art and it is within the skill of the artisan to select an appropriate one. Upon expression in a tumor cell of the β-catenin binding portion of APC, β-catenin will be bound and titrated away from binding to Tcf-4, thus preventing unregulated expression of the CRT target genes. Similarly, a polypeptide portion of APC containing the β-catenin binding site can be administered to cells to perform a titration of β-catenin. Techniques for such administration to cells is well known in the art. Cells which are treated with either the polynucleotide or the polypeptide can be used to study the interaction between APC and β-catenin, and for developing drugs which interfere with such binding.

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples which are provided herein for purposes of illustration only, and are not intended to limit the scope of the invention.

EXAMPLE 1

This example identifies Tcf-4 as the expressed family member in colorectal epithelial cells and provides the complete sequence of the cloned cDNA.

There are four known members of the Tcf/Lef family in mammals: the lymphoid-specific factors Tcf- I and Lef- 1 (7,8), and the less well characterized Tcf-3 and 4(9). We performed a qualitative Reverse Transcriptase-Polymerase Chain Reaction assay for expression of the four

Tcf/Lef genes on 43 colon tumor cell lines. While most colon cell lines expressed more than one of the genes, only hTcf-4 mRNA was expressed in essentially all lines.

We then screened a human fetal CDNA library and retrieved clones encoding full-length hTcf-4 (Fig. 1). A genomic fragment encoding, the HMG box region of hTcf-4 (7) was used to probe a human 12 week-fetal cDNA library in Lambda GT-11. Positive clones were subcloned into pBluescriptSK and sequenced. See SEQ ID NOs: 1 and 3. The predicted sequence of hTcf-4 was most similar to that of hTcf-1. Alternative splicing yielded two COOH-termini that were conserved between hTcf- I and hTcf-4. The NH_2-terminus, which in hTcf- 1, mLef-1 and *Xenopus* TCF-3 mediates binding to β-catenin (6), was also conserved in hTcf-4. Northern blot analysis of selected colon carcinoma cell lines revealed high-level expression of *hTcf-4* (Fig. 2A). Northern blot hybridizations (7) were performed with full-length *hTcf-1, hLef-I and hTcf-4* cDNA. Colon epithelial cells were freshly prepared from a mucosal preparation dissected from a healthy surgical colon sample. The sample was minced, and incubated with 1 mM dithiothreitol (DTT) in Hanks' medium to remove mucus. Single-cell suspensions were prepared by incubation at RT in 0.75 mM EDTA in Hanks' medium. Epithelial cells were separated from lymphocytes by Percoll gradient centrifugation.

As evidenced by in situ hybridization (Fig. 2, B and C) and Northern blotting (Fig. 2A), *hTcf-4* MRNA was readily detectable in normal colonic epithelium, whereas *hTcf-I and hLe*f-I were not detectable. In situ hybridization of 6 μ frozen sections of healthy colon biopsy samples were performed as described(10). *hTcf-4 cDNA* encoding amino acids 200 to 310 was amplified and labeled with Dig-11-dUTP (Boehringer Mannheim, Germany) by PCR. After hybridization and washing, the sections were sequentially incubated with mouse anti-Dig antibody (Boehringer) and a horseradish peroxidase conjugated rabbit antibody to mouse immunoglobulin

(Dako, Glostrup, Denmark). The signal was visualized with diaminobenzidine, which produces a reddish-brown precipitate. Blue counterstining was performed with haematoxyline.

EXAMPLE 2

This example demonstrates the interaction of Tcf-4 and β-catenin and their function as a transcriptional activating factor.

To investigate whether hTcf-4 functionally interacts with β-catenin, we used two sets of reporter constructs in a β -catenin-Tcf reporter gene assay (7). One contained three copies of the optimal Tcf motif CCTTTGATC, or three copies of the mutant motif CCTTTGGCC, upstream of a minimal *c-Fos* promoter driven-luciferase expression (PTOPFLASH and PFOPFLASH). The second set contained three copies of the optimal motif, or three copies of the mutant motif, upstream of a minimal Herpes virus thymidine kinase promoter driven-Chloramphenicol Acetyl Transferase (CAT) expression (PTOPCAT and PFOPCAT, respectively). Reporter gene assays were performed as in (7). In brief, 2×10^6 cells were transfected with plasmids by electroporation. After 24 hours, cells were harvested and lysed in 1 mM DTT, 1 % Triton X-100, 15 % glycerol, 25 mM Tris pH 7.8 and 8 mM $MgC1_2$. cDNAs encoding Myc-tagged versions of β-catenin and hTcf-4 were inserted into the mammalian expression vector pCDNA (Invitrogen). PCATCONTROL, encoding the CAT enzyme under the control of the SV40 promoter, was purchased from Promega.

Epitope-tagged hTcf-4 and a deletion mutant lacking, the NH_2-terminal 30 amino acids (ΔNhTcf-4) were cloned into the expression vector pCDNA. Transient transfections were performed in a murine B cell line (IIA1.6), that does not express any of the Tcf genes (6).

The TOPFLASH reporter was strongly transcribed upon cotransfection with the combination of β-catenin and hTcf-4 plasmids, but not with the individual plasmids or with the combination of β-catenin and ΔNhTcf-4

plasmids. No enhanced transcription was detected in cells transfected with the negative control PFOPFLASH (Fig. 3A). These results show that interaction of the NH_2-terminus of hTcf-4 with β-catenin results in transcriptional activation.

EXAMPLE 3

This example demonstrates the functional regulation of CRT transcriptional activation by wild-type APC.

In three $APC^{-/-}$ carcinoma cell lines, SW480, SW620 and DLD-1 (Fig. 3B), the PTOPFLASH reporter was 5-20 fold more actively transcribed than PFOPFLASH. Importantly, transfection of SW480 cells with the reporter gene and an APC-expression vector abrogated the transcriptional activity in a dose-dependent manner (Fig. 3B). In contrast APC had no effect on a cotransfected internal control (pCATCONTROL), or on the basal transcription of PFOPFLASH (Fig. 3B). The use of PTOPCAT and PFOPCAT instead of PTOPFLASH and PFOPFLASH led to comparable observations. The constitutive transcriptional activity of Tcf reporter genes in $APC^{-/-}$ colon carcinoma cells was in stark contrast to the inactivity of these genes in non-colonic cell lines, including IIA1.6 B cells (Fig. 3A), the C57MG breast carcinoma cell line; the Jurkat and BW5147 T cell lines; the Daudi and NS1 B cell lines; the K562 erythromyeloid cell line; the HeLa cervical carcinoma line; the HepG2 hepatoma cell line; 3T3, 3T6, and Rat-I fibroblasts; and the kidney derived SV40-transformed COS cell line (7,16).

EXAMPLE 4

This example demonstrates that a functional β-catenin-hTcf-4 complex exists constitutively in $APC^{-/-}$ cells.

We used HT29-$APC^{-/-}$ colon carcinoma cells (12), in which APC is controlled by a metallothionein promoter. Induction by Zn^{++} restores wild-type levels of APC, and leads to apoptosis (12). HT29-Gal cells which carry a Zn^{++}-inducible LacZ gene were used as a control. The only Tcf family member expressed in HT29 is hTcf-4 (Fig. 2C). In nuclear extracts

from uninduced HT29 derived transfectants, we readily detected hTcf-4 by gel retardation (Fig. 4). An additional band of slightly slower mobility was also observed. The addition of a β-catenin antibody resulted in the specific retardation of the latter band, indicating that it represented a β-catenin-hTcf-4 complex (Fig. 4) (12). After Zn^{++} induction for 20 hours, the β-catenin-hTcf-4 complex was diminished sixfold relative to uncomplexed hTcf-4 in HT29-APC1, while no significant change was observed in HT29-Gal cells (Fig. 4). Importantly, the overall levels of cellular β-catenin do not change during the induction period in HT29-APC1 cells (12).

Gel retardation assays were performed as described elsewhere (7). Extracts were prepared from intact nuclei that were washed four times to avoid contamination with cytoplasmic β-catenin. As the optimal Tcf/Lef probe, we used a double-stranded 15-mer CCCTTTGATCTTACC; the control probe was CCCTTTGGCCTTACC. (All oligonucleotides were from Isogene, Holland). The β-catenin antibody was purchased from Transduction Laboratories (Lexington, KY). A typical binding reaction contained 3 μg nuclear protein, 0. 1 ng radiolabeled probe, 100 ng of dIdC, in 25 μl of binding buffer (60 mm KCl, 1 mM EDTA, 1 mM DTT, 10% glycerol). Samples were incubated for 20 min at room temperature, antibody was added, and the samples incubated 20 min further.

On the basis of these data, we propose the following model. In normal colonic epithelium hTcf-4 is the only expressed member of the Tcf family. The interaction of β-catenin with hTcf-4 is regulated by APC. When appropriate extracellular signals are delivered to an epithelial cell, β-catenin accumulates in a form that is not complexed with GSK3β-APC, and that enables its nuclear transport and association with hTcf-4. The HMG domain of hTcf-4 binds in a sequence-specific fashion to the regulatory sequences of specific target genes; β-catenin supplies a transactivation domain. Thus, transcriptional activation of target genes occurs only when hTcf-4 is associated with β-catenin. The hTcf-4 target genes remain to be

identified. However, the link with APC and catenin suggests that these genes may participate in the generation and turnover of epithelial cells. Upon loss of wild-type APC, monomeric β-catenin accumulates in the absence of extracellular stimuli, leading to uncontrolled transcription of the hTcf-4 target genes. The apparent *de novo* expression of other members of the *Tcf* family in some colon carcinoma cell lines might lead to a further deregulation of Tcf target gene expression by the same mechanism. The control of β-catenin -Tcf signaling is likely to be an important part of the gatekeeper function of APC (19), and its disruption an early step in malignant transformation.

EXAMPLE 5

This example demonstrates that mutant APC protein does not regulate CRT and that a complete set of 20-AA repeats in APC is required to mediate inhibition of CRT.

We tested four APC mutants (Fig. 5A) for their ability to inhibit β-catenin/Tcf-regulated transcription (CRT) in transfection assays. The first mutant, APC331Δ represents a type of mutation found in the germline of Familial Adenomatous Polyposis (FAP) patients as well as in sporadic tumors (15). The APC331Δ protein is truncated at codon 331, amino-terminal to the three 15-amino-acid (AA) β-catenin-binding repeats between codons 1020 and 1169. The second mutant, APC1309Δ, is the most common germline *APC* mutation (15), a 5-bp deletion that produces a frameshift at codon 1309 and truncation of the protein. The APC1309Δ protein retains the 15-AA β-catenin repeats but lacks the seven 20-AA repeats between codons 1323 and 2075 that have been implicated in binding and phosphorylation of β-catenin (*18*). The third mutant, APC1941Δ, represents one of the most distal somatic mutations observed in colorectal tumors (*25*). The APC1941Δ protein is truncated at codon 1941 and therefore contains the 15-AA repeats and all but the last two 20-AA repeats.

Finally, APC2644Δ represents a germline mutation resulting from a 4-bp deletion in codon 2644. Patients with this type of unusual carboxyl-terminal mutation develop few polyps (attenuated polyposis) but have pronounced extracolonic disease, particularly desmoid tumors (*26*).

Each of the *APC* mutants was cotransfected with a CRT reporter into the SW480 colorectal cancer cell line. SW480 cells have truncated APC and constitutively active CRT which can be suppressed by exogenous WT APC. Although all four mutants produced comparable levels of APC protein after transfection, they varied in their CRT inhibitory activity. The three mutants found in patients with typical polyposis or cancer were markedly deficient in inhibition of CRT (Fig. 5B). The reduced activity of APC1309Δ and APC1941Δ suggests that β-catenin binding is not sufficient for APC-mediated inhibition of CRT and that the complete set of 20-AA repeats is required. Interestingly, the inhibitory activity of the APC2644Δ mutant associated with attenuated polyposis was comparable to that of WT APC (Fig. 5B), suggesting that the DLG-binding domain at the carboxyl-terminus of APC is not required for down-regulation of CRT.

WT and mutant *APC* constructs (2 μg) were transfected into 293, SW480, and HCT116 cells using Lipofectamine (GIBCO/BRL, Gaithersburg). Protein was harvested 24 hours later and subjected to immunoblot analysis with APC monoclonal antibody FE9 (*23*). In HCT116 and 293 cells, exogenous WT APC comigrated with the endogenous APC. In SW480 cells, APC1309Δ comigrated with the endogenous mutant APC. In all other cases, the nonfunctional APC constructs (APC331Δ, APC 1309Δ, and APC1941Δ) produced as much or more protein than the CRT-functional forms of APC (APC WT and APC 2644Δ).

EXAMPLE 6

This example demonstrates that other components of the APC-regulatory pathway are affected in some cancer cells.

We evaluated CRT in two colorectal tumor cell lines (HCT116 and SW48) that express full-length APC (Fig. 6A). Both HCT116 and SW48 displayed constitutively active CRT and, in contrast to cell lines with truncated APC (DLD1 and SW480), this activity was not inhibited by exogenous WT APC (Fig. 5B, 6B). Other (noncolorectal cancer) cell lines expressing WT APC do not display constitutive CRT activity. These transfection results suggested that the constitutive CRT in HCT116 and SW48 might be due to an altered downstream component of the APC tumor suppressor pathway.

EXAMPLE 7

This example demonstrates a defect in the gene encoding β-catenin in some cancer cells, which affects CRT.

We evaluated the status of a likely candidate for a downstream component of the APC tumor suppressor pathway, β-catenin, in the same four lines. All four lines expressed similar amounts of apparently intact β-catenin, as assessed by immunoblots (Fig. 7A). However, sequence analysis revealed that both HCT116 and SW48 harbored mutations in the β-catenin gene (*CTNNB1*) (Fig. 7B). HCT116 had a 3-bp deletion that removed one AA (Ser-45), and SW48 had a C to A missense mutation that changed Ser-33 to Tyr. Analysis of paraffin-embedded archival tissue from the HCT116 patient confirmed the somatic nature of this mutation and its presence in the primary tumor prior to culture. Interestingly, both mutations affected serines that have been implicated in the downregulation of β-catenin through phosphorylation by the ZW3/GSK3β kinase in *Xenopus* embryos (Fig. 7C) (*27,28*).

Genomic DNA was isolated from paraffin-embedded normal and tumor tissue from the patient from whom the HCT116 cell line was derived. A 95 bp PCR product encompassing the mutation was then amplified by PCR and directly sequenced using THERMOSEQUENASE (Amersham). The 3 bp deletion was observed in tumor but not in normal tissue.

To test the generality of this mutational mechanism, we evaluated five primary colorectal cancers in which sequencing of the entire coding region of *APC* revealed no mutations (*25*). Three of these five tumors were found to contain *CTNNB1* mutations (S45F, S45F, and T44A) that altered potential ZW3/GSK3β phosphorylation sites (Fig. 7C). Each mutation appeared to affect only one of the two *CTNNB1* alleles and to be somatic.

Genomic DNA was isolated from frozen-sectioned colorectal cancers and a 1001 bp PCR product containing exon 3 of *CTNNB1* was then amplified by PCR and directly sequenced using ThermoSequenase (Amersham). An ACC to GCC change at codon 41 (T41A) and a TCT to TTT at codon 45 (S45F) was observed in one and two tumors, respectively.

EXAMPLE 8

This example demonstrates dominant mutations of *CTNNB1* that render CRT insensitive to the effects of WT APC.

Because the β-catenin mutations were heterozygous, we hypothesized that the mutations might exert a dominant effect, rendering a fraction of cellular β-catenin insensitive to APC-mediated down regulation. To test this notion, we performed gel shift analyses with nuclear extracts from untransfected HCT116 cells. In contrast to noncolorectal cancer cell lines with intact *APC*, HCT116 cells contained a β-catenin/Tcf complex that gel-shifted an optimized Tcf-binding oligonucleotide, and this complex supershifted with anti-β-catenin (Fig. 8A). We also constructed β-catenin expression vectors and compared the biologic activity of the mutant β-catenin from HCT116 (β-Cat Δ45) and SW48 (β-Cat S33Y) with that of

their WT counterpart. For these experiments, we used the 293 kidney epithelial cell line as it is highly transfectable, exhibits low endogenous CRT, and contains a high level of endogenous APC (Fig. 6A). In the presence of endogenous APC, both mutant β-catenins were at least 6-fold more active than the WT protein and this activity was inhibited by dominant-negative hTcf-4 (Fig. 8B).

Together, these results indicate that disruption of APC-mediated regulation of CRT is critical for colorectal tumorigenesis. This is most commonly achieved by recessive inactivating mutations of both *APC* alleles but, as shown here, can also be achieved by dominant mutations of *CTNNB1* that render CRT insensitive to the effects of WT APC. Our results suggest that APC inhibition of CRT requires phosphorylation of β-catenin at multiple sites. These potential phosphorylation sites are consistent with the known specificity of ZW3/GSK3β (*29*) a serine kinase that negatively regulates β-catenin in *Xenopus* and *Drosophila* cells (*27*) and that interacts with APC and β-catenin in mammalian cells (*23*). These results also suggest a functional basis for the occasional *CTNNB1* mutations observed in other tumor types (*30*) and illustrate how a critical pathway in human disease can be illuminated by the discovery of mutations in different components of the pathway. The next step in understanding *APC* function will be the identification of the genes that are activated by hTcf-4/β-catenin complexes and inhibited by WT APC. These genes are likely to be related to APC's ability to induce apoptosis in colorectal cancer cells (*31*).

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SEQUENCE LISTING

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SEQ ID NO: 6 Tcf mutant motif

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SEQ ID NO: 7 APC amino acid

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TTRMDHETAS VLSSSSTHSA

310 320 330 340 350 360
PRRLTSHLGT KVEMVYSLLS MLGTHDKDDM SRTLLAMSSS
QDSCISMRQS GCLPLLIQLL

370 380 390 400 410 420

HGNDKDSVLL GNSRGSKEAR ARASAALHNI IHSQPDDKRG
RREIRVLHLL EQIRAYCETC

430 440 450 460 470 480

WEWQEAHEPG MDQDKNPMPA PVEHQICPAV CVLMKLSFDE
EHRHAMNELG GLQAIAELLQ

490 500 510 520 530 540

VDCEMYGLTN DHYSITLRRY AGMALTNLTF GDVANKATLC
SMKGCMRALV AQLKSESEDL

550 560 570 580 590 600

QQVIASVLRN LSWRADVNSK KTLREVGSVK ALMECALEVK
KESTLKSVLS ALWNLSAHCT

610 620 630 640 650 660

ENKADICAVD GALAFLVGTL TYRSQTNTLA IIESGGGILR
NVSSLIATNE DHRQILRENN

670 680 690 700 710 720

CLQTLLQHLK SHSLTIVSNA CGTLWNLSAR NPKDQEALWD
MGAVSMLKNL IHSKHKMIAM

730 740 750 760 770 780

GSAAALRNLM ANRPAKYKDA NIMSPGSSLP SLHVRKQKAL
EAELDAQHLS ETFDNIDNLS

790 800 810 820 830 840

PKASHRSKQR HKQSLYGDYV FDTNRHDDNR SDNFNTGNMT
VLSPYLNTTV LPSSSSSRGS

850 860 870 880 890 900

LDSSRSEKDR SLERERGIGL GNYHPATENP GTSSKRGLQI
STTAAQIAKV MEEVSAIHTS

910 920 930 940 950 960

QEDRSSGSTT ELHCVTDERN ALRRSSAAHT HSNTYNFTKS
ENSNRTCSMP YAKLEYKRSS

970 980 990 1000 1010 1020

NDSLNSVSSS DGYGKRGQMK PSIESYSEDD ESKFCSYGQY
PADLAHKIHS ANHMDDNDGE

1030 1040 1050 1060 1070 1080

LDTPINYSLK YSDEQLNSGR QSPSQNERWA RPKHIIEDEI
KQSEQRQSRN QSTTYPVYTE

1090 1100 1110 1120 1130 1140

STDDKHLKFQ PHFGQQECVS PYRSRGANGS ETNRVGSNHG
INQNVSQSLC QEDDYEDDKP

1150 1160 1170 1180 1190 1200

TNYSERYSEE EQHEEEERPT NYSIKYNEEK RHVDQPIDYS
LKYATDIPSS QKQSFSFSKS

1210 1220 1230 1240 1250 1260

SSGQSSKTEH MSSSSENTST PSSNAKRQNQ LHPSSAQSRS
GQPQKAATCK VSSINQETIQ

1270 1280 1290 1300 1310 1320

TYCVEDTPIC FSRCSSLSSL SSAEDEIGCN QTTQEADSAN
TLQIAEIKEK IGTRSAEDPV

1330 1340 1350 1360 1370 1380

SEVPAVSQHP RTKSSRLQGS SLSSESARHK AVEFSSGAKS
PSKSGAQTPK SPPEHYVQET

1390 1400 1410 1420 1430 1440

PLMFSRCTSV SSLDSFESRS IASSVQSEPC SGMVSGIISP
SDLPDSPGQT MPPSRSKTPP

1450 1460 1470 1480 1490 1500

PPPQTAQTKR EVPKNKAPTA EKRESGPKQA AVNAAVQRVQ
VLPDADTLLH FATESTPDGF

1510 1520 1530 1540 1550 1560

SCSSSLSALS LDEPFIQKDV ELRIMPPVQE NDNGNETESE
QPKESNENQE KEAEKTIDSE

1570 1580 1590 1600 1610 1620

KDLLDDSDDD DIEILEECII SAMPTKSSRK AKKPAQTASK

LPPPVARKPS QLPVYKLLPS

1630 1640 1650 1660 1670 1680

QNRLQPQKHV SFTPGDDMPR VYCVEGTPIN FSTATSLSDL

TIESPPNELA AGEGVRGGAQ

1690 1700 1710 1720 1730 1740

SGEFEKRDTI PTEGRSTDEA QGGKTSSVTI PELDDNKAEE

GDILAECINS AMPKGKSHKP

1750 1760 1770 1780 1790 1800

FRVKKIMDQV QQASASSSAP NKNQLDGKKK KPTSPVKPIP

QNTEYRTRVR KNADSKNNLN

1810 1820 1830 1840 1850 1860

AERVFSDNKD SKKQNLKNNS KDFNDKLPNN EDRVRGSFAF

DSPHHYTPIE GTPYCFSRND

1870 1880 1890 1900 1910 1920

SLSSLDFDDD DVDLSREKAE LRKAKENKES EAKVTSHTEL

TSNQQSANKT QAIAKQPINR

1930 1940 1950 1960 1970 1980

GQPKPILQKQ STFPQSSKDI PDRGAATDEK LQNFAIENTP

VCFSHNSSLS SLSDIDQENN

1990 2000 2010 2020 2030 2040

NKENEPIKET EPPDSQGEPS KPQASGYAPK SFHVEDTPVC

FSRNSSLSSL SIDSEDDLLQ

2050 2060 2070 2080 2090 2100

ECISSAMPKK KKPSRLKGDN EKHSPRNMGG ILGEDLTLDL

KDIQRPDSEH GLSPDSENFD

2110 2120 2130 2140 2150 2160

WKAIQEGANS IVSSLHQAAA AACLSRQASS DSDSILSLKS

GISLGSPFHL TPDQEEKPFT

2170 2180 2190 2200 2210 2220

SNKGPRILKP GEKSTLETKK IESESKGIKG GKKVYKSLIT
GKVRSNSEIS GQMKQPLQAN

2230 2240 2250 2260 2270 2280

MPSISRGRTM IHIPGVRNSS SSTSPVSKKG PPLKTPASKS
PSEGQTATTS PRGAKPSVKS

2290 2300 2310 2320 2330 2340

ELSPVARQTS QIGGSSKAPS RSGSRDSTPS RPAQQPLSRP
IQSPGRNSIS PGRNGISPPN

2350 2360 2370 2380 2390 2400

KLSQLPRTSS PSTASTKSSG SGKMSYTSPG RQMSQQNLTK
QTGLSKNASS IPRSESASKG

2410 2420 2430 2440 2450 2460

LNQMNNGNGA NKKVELSRMS STKSSGSESD RSERPVLVRQ
STFIKEAPSP TLRRKLEESA

2470 2480 2490 2500 2510 2520

SFESLSPSSR PASPTRSQAQ TPVLSPSLPD MSLSTHSSVQ
AGGWRKLPPN LSPTIEYNDG

2530 2540 2550 2560 2570 2580

RPAKRHDIAR SHSESPSRLP INRSGTWKRE HSKHSSSLPR
VSTWRRTGSS SSILSASSES

2590 2600 2610 2620 2630 2640

SEKAKSEDEK HVNSISGTKQ SKENQVSAKG TWRKIKENEF
SPTNSTSQTV SSGATNGAES

2650 2660 2670 2680 2690 2700

KTLIYQMAPA VSKTEDVWVR IEDCPINNPR SGRSPTGNTP
PVIDSVSEKA NPNIKDSKDN

2710 2720 2730 2740 2750 2760

QAKQNVGNGS VPMRTVGLEN RLNSFIQVDA PDQKGTEIKP
GQNNPVPVSE TNESSIVERT

2770 2780 2790 2800 2810 2820

PFSSSSSSKH SSPSGTVAAR VTPFNYNPSP RKSSADSTSA RPSQIPTPVN NNTKKRDSKT

2830 2840 2850 2860 2870 2880

DSTESSGTQS PKRHSGSYLV TSV*KRGRMK LRKFYVNYNC YIDILFQMKL *KTEKFCK*V

2890 2900 2910 2920 2930 2940

*FLLEGFCSG SHI**YTLSS LVLFWEALLM VRKKIVKPSM FVQYVLHVFK VAPIPTSFNY

2950 2960 2970 2980 2990 3000

CLS*NNEHYR *KI*YIAVIN HF*IIN*LNL HQGKIGIYAK KNVF...... ....

SEQ ID NO: 8 Tcf-1B amino acid

SEQ ID NO: 9 Tcf-1E amino acid

CLAIMS

1. An intron-free DNA molecule encoding Tcf-4 protein as shown in SEQ ID NO: 2 or 4.

2. The DNA molecule of claim 1 which has the nucleotide sequence of SEQ ID NO: 1 or 3.

3. An isolated Tcf-4 protein, substantially free of other human proteins, having a sequence as shown in SEQ ID NO: 2 or 4.

4. A method of determining the presence or absence in a cell of wild-type APC or a downstream protein in the APC transcription regulatory pathway, comprising the steps of:

introducing a Tcf-responsive reporter gene into the cell; and

measuring transcription of said reporter gene; wherein a cell which supports active transcription of said reporter gene does not have wild-type APC or a downstream protein in the APC transcription regulatory pathway.

5. A method of determining the presence or absence in a cell of wild-type APC, comprising the steps of:

contacting a Tcf-responsive reporter gene with a lysate of the cell; and

measuring transcription of said reporter gene; wherein a lysate which inhibits said transcription has wild-type APC.

6. A method of identifying candidate drugs for use in FAP patients, patients with APC or β-catenin mutations, or patients with increased risk of developing cancer, comprising the steps of:

contacting a cell having no wild-type APC or a mutant β-catenin with a test compound;

measuring transcription of a Tcf-responsive reporter gene, wherein a test compound which inhibits the transcription of the reporter gene is a candidate drug for cancer therapy.

7. The method of claim 6 wherein the cell produces an APC protein defective in β-catenin binding or regulation.

8. The method of claim 6 wherein the cell produces a β-catenin protein which is super-active, or which is defective in APC binding or resistant to APC regulation.

9. The method of claim 6 wherein the cell produces no detectable APC protein.

10. A method of identifying candidate drugs for use in for use in FAP patients, patients with APC or β-catenin mutations, or patients with increased risk of developing cancer, comprising the steps of:

contacting a Tcf-responsive reporter gene with a test compound under conditions in which the reporter gene is transcribed in the absence of the test compound; and

measuring transcription of the Tcf-responsive reporter gene; wherein a test compound which inhibits said transcription is a candidate drug for cancer therapy.

11. The method of claim 10 wherein the step of contacting is performed in the presence of a lysate of a cell which has no wild-type APC.

12. The method of claim 10 wherein the step of contacting is performed in the presence of a lysate of a cell which has a mutant β-catenin defective in APC binding or resistant to APC regulation or which is super-active.

13. The method of claim 11 wherein the cell produces an APC protein defective in β-catenin binding or regulation.

14. A method for diagnosing cancer in a sample suspected of being neoplastic, comprising the steps of:

comparing a CTNNB sequence found in the sample to a second CTNNB sequence found in a normal tissue, wherein a difference between the first and second sequence is an indicator of cancer.

15. The method of claim 14 wherein the sequences are nucleotide sequences.

16. The method of claim 14 wherein the sequences are amino acid sequences.

17. The method of claim 14 wherein the normal tissue is isolated from the same human as the sample.

18. The method of claim 14 wherein the cancer is selected from the group consisting of: colorectal cancer, thyroid cancer, brain cancer, medulloblastoma, desmoid tumor, osteoma, breast cancer, and head and neck cancer.

19. The method of claim 14 wherein the step of comparing is preceded by the step of:

comparing a first APC sequence found in the sample to a second APC sequence found in a normal tissue.

20. The method of claim 19 where no differences are detected between the first and second APC sequences.

21. The method of claim 14 wherein no APC mutations have been detected in the sample.

22. A method for treating a patient with colorectal cancer or other cancer associated with FAP, the method comprising the step of:

administering to the patient a nucleotide sequence comprising a portion of the APC coding sequence, said portion consisting of the β-catenin binding site.

23. A method for treating a patient with colorectal cancer or other cancer associated with FAP, the method comprising the step of:

administering to the patient a polypeptide comprising a portion of the APC coding sequence, said portion consisting of the β-catenin binding site.

24. A method of identifying candidate drugs for use in FAP patients or patients with increased risk of developing cancer, comprising the steps of:

contacting a test compound with β-catenin and Tcf-4 under conditions in which β-catenin and Tcf-4 bind to each other; and

determining whether the test compound inhibits the binding of β-catenin and Tcf-4, a test compound which inhibits the binding being a candidate for cancer therapy or prophylaxis.

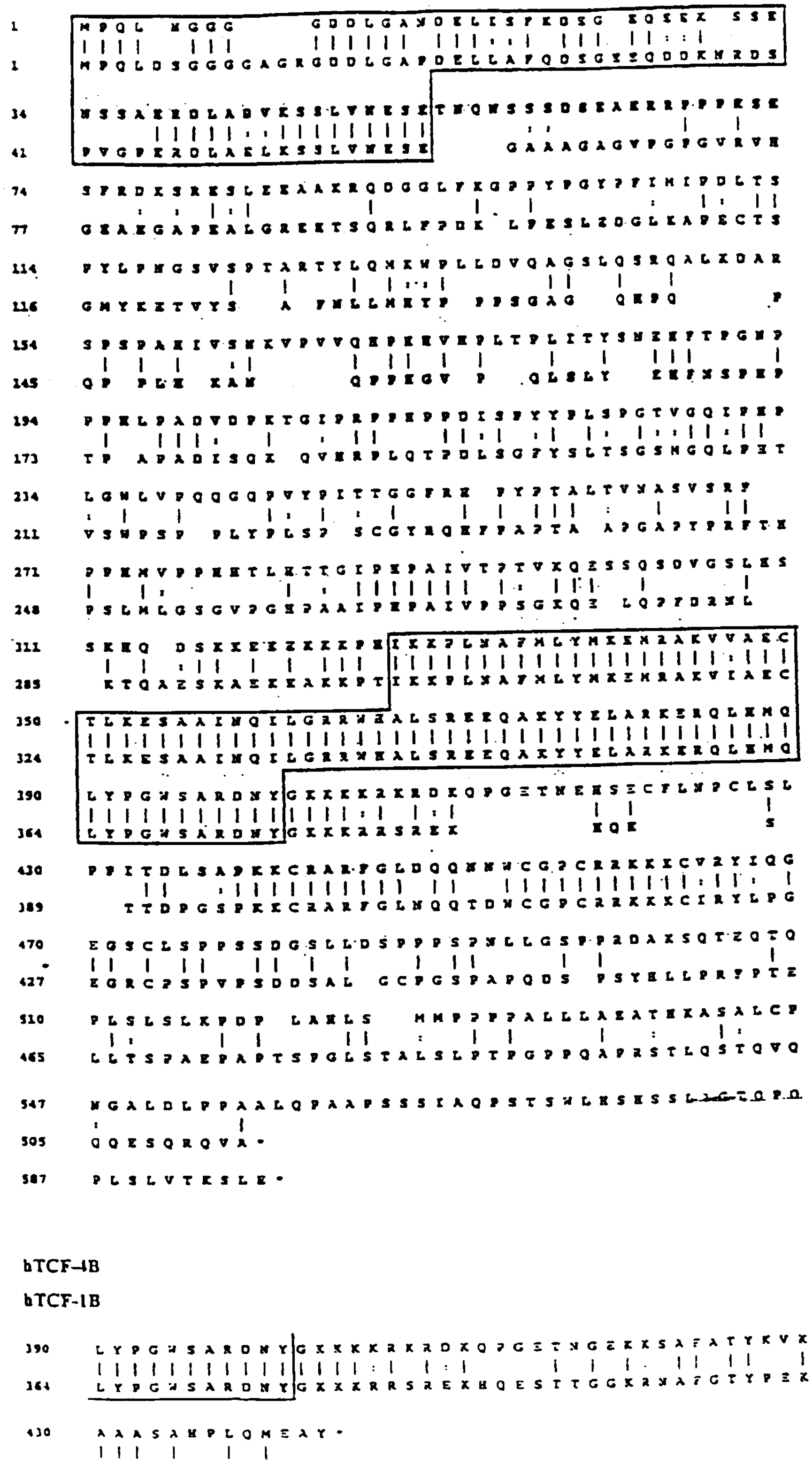


FIGURE 1

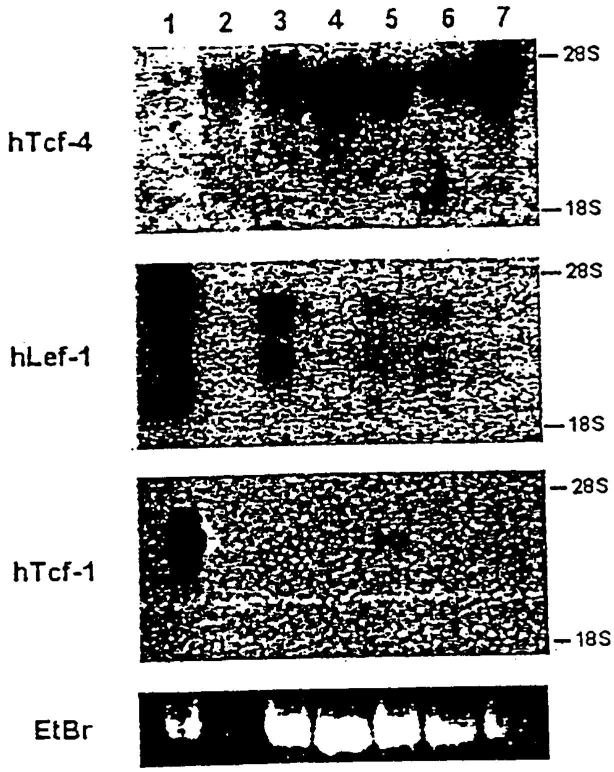


FIGURE 2A

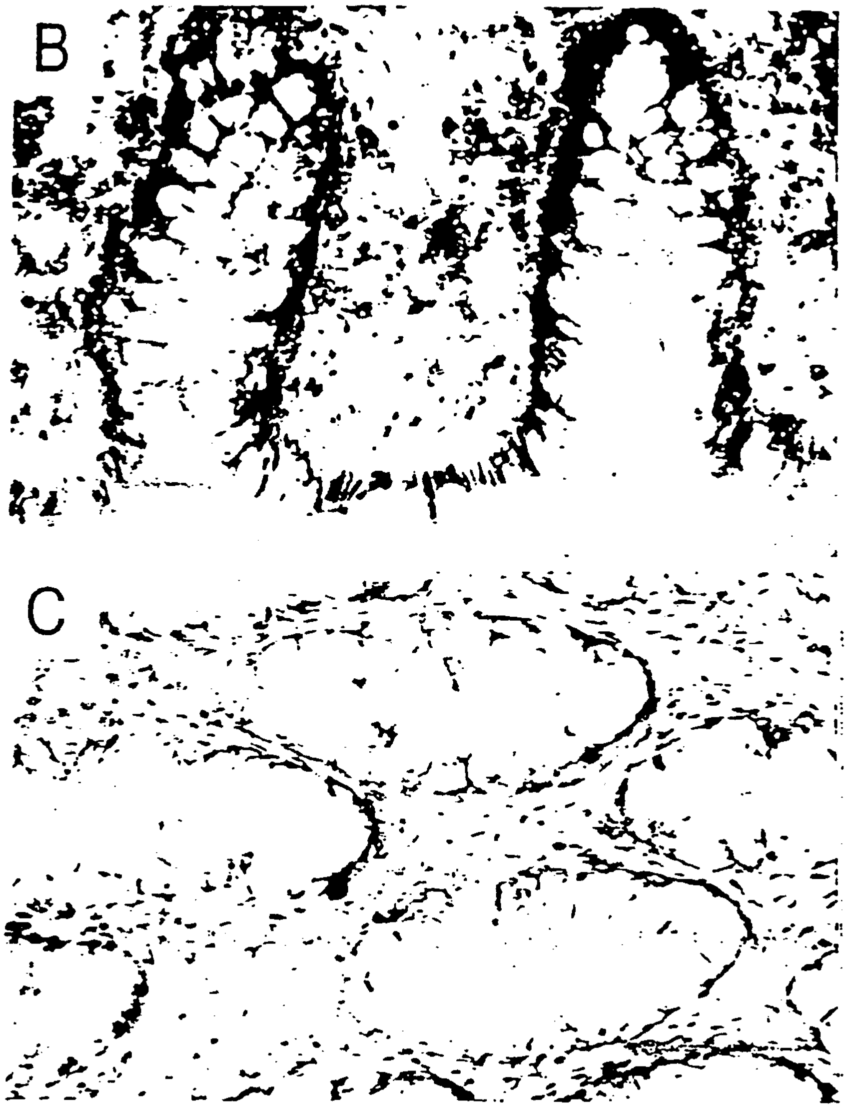


FIGURE 2 B & C

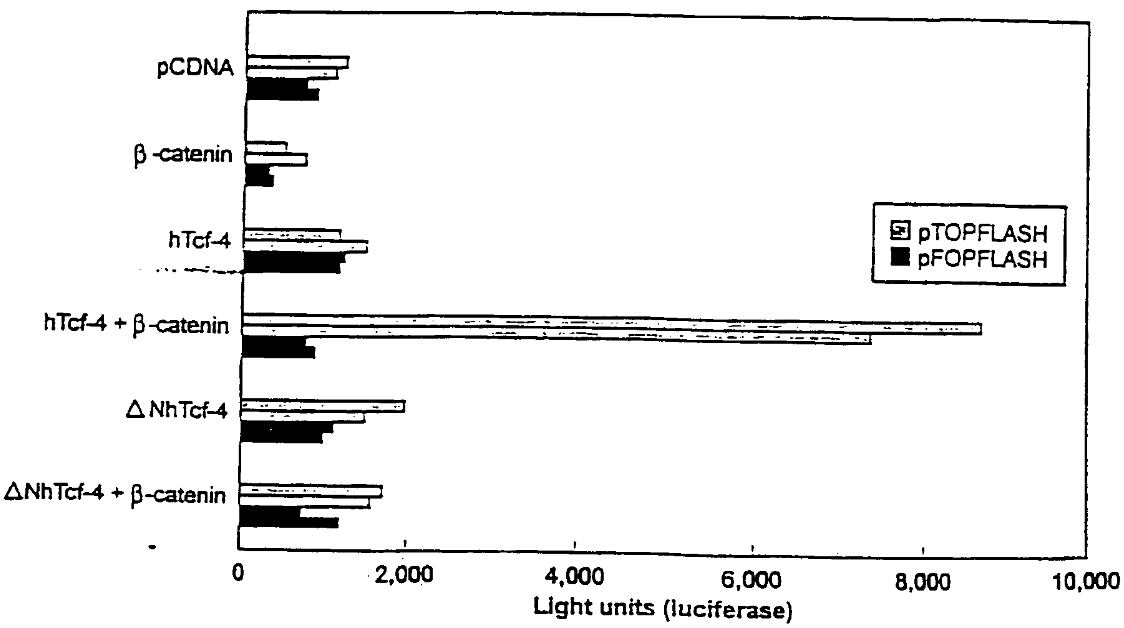

pCDNA
β-catenin
hTcf-4
hTcf-4 + β-catenin
Δ NhTcf-4
ΔNhTcf-4 + β-catenin
pTOPFLASH
pFOPFLASH
0
2,000
4,000
6,000
8,000
10,000
Light units (luciferase)

FIGURE 3A

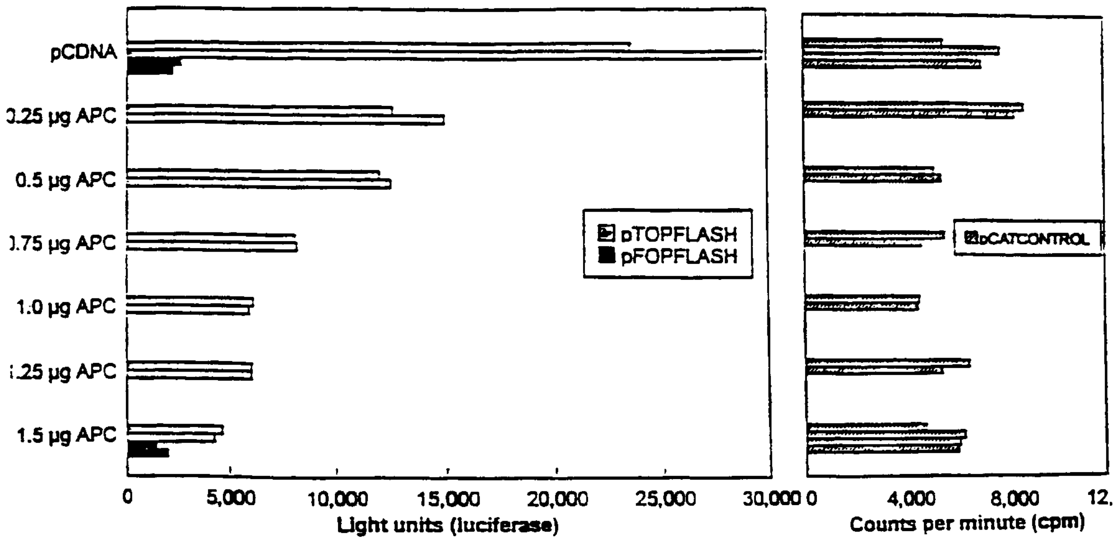

pCDNA
0.25 µg APC
0.5 µg APC
0.75 µg APC
1.0 µg APC
1.25 µg APC
1.5 µg APC
pTOPFLASH
pFOPFLASH
pCATCONTROL
0
5,000
10,000
15,000
20,000
25,000
30,000
Light units (luciferase)
0
4,000
8,000
12,
Counts per minute (cpm)

FIGURE 3B

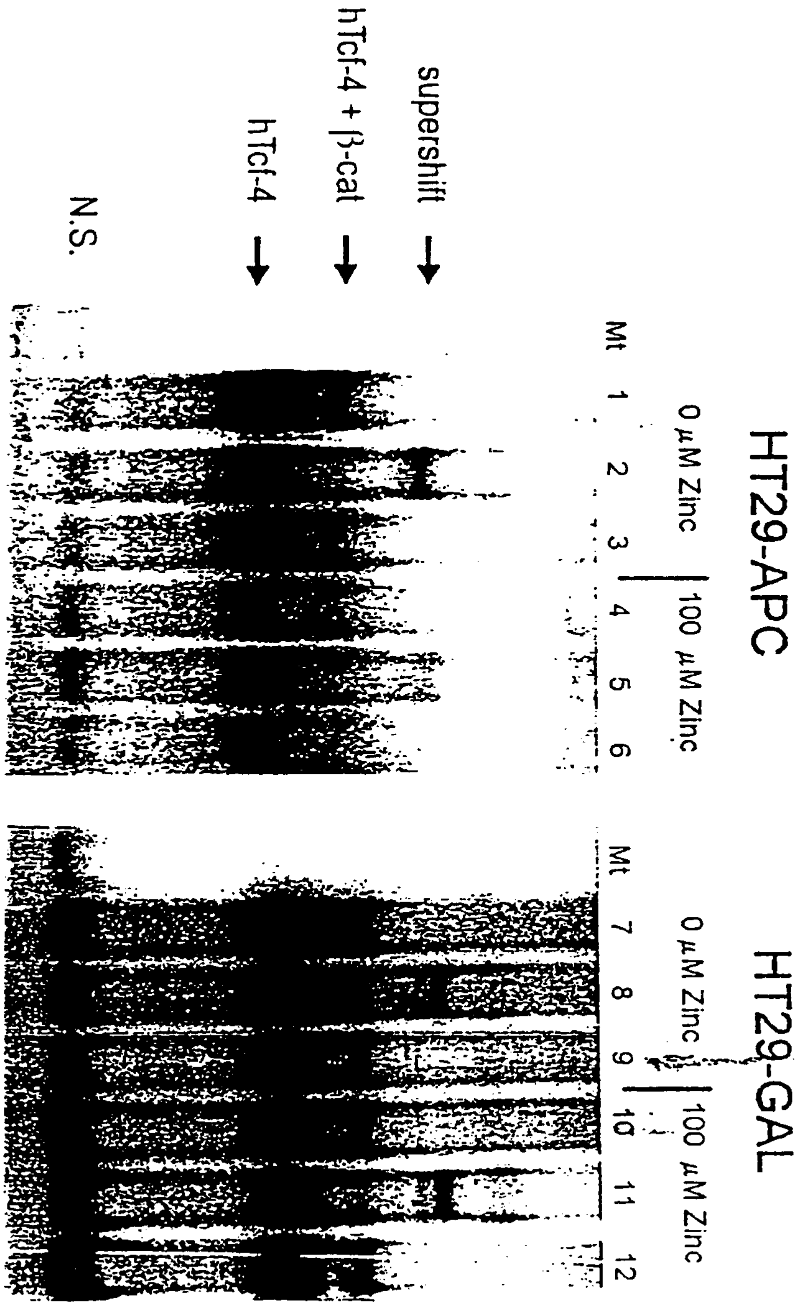

HT29-APC
0 μM Zinc
100 μM Zinc
Mt 1 2 3 4 5 6
HT29-GAL
0 μM Zinc
100 μM Zinc
Mt 7 8 9 10 11 12
supershift
hTcf-4 + β-cat
hTcf-4
N.S.

FIGURE 4


APC331Δ
APC1309Δ
APC1941Δ
APC2644Δ
APC (WT)
Sequence Features
Amino Acids
Heptad Repeats
ARM Repeats
15 AA Repeats
20 AA Repeats
Catenin Binding Repeats
Basic Region
TXV-COOH
1
500
1000
1500
2000
2500
2843

FIGURE 5A

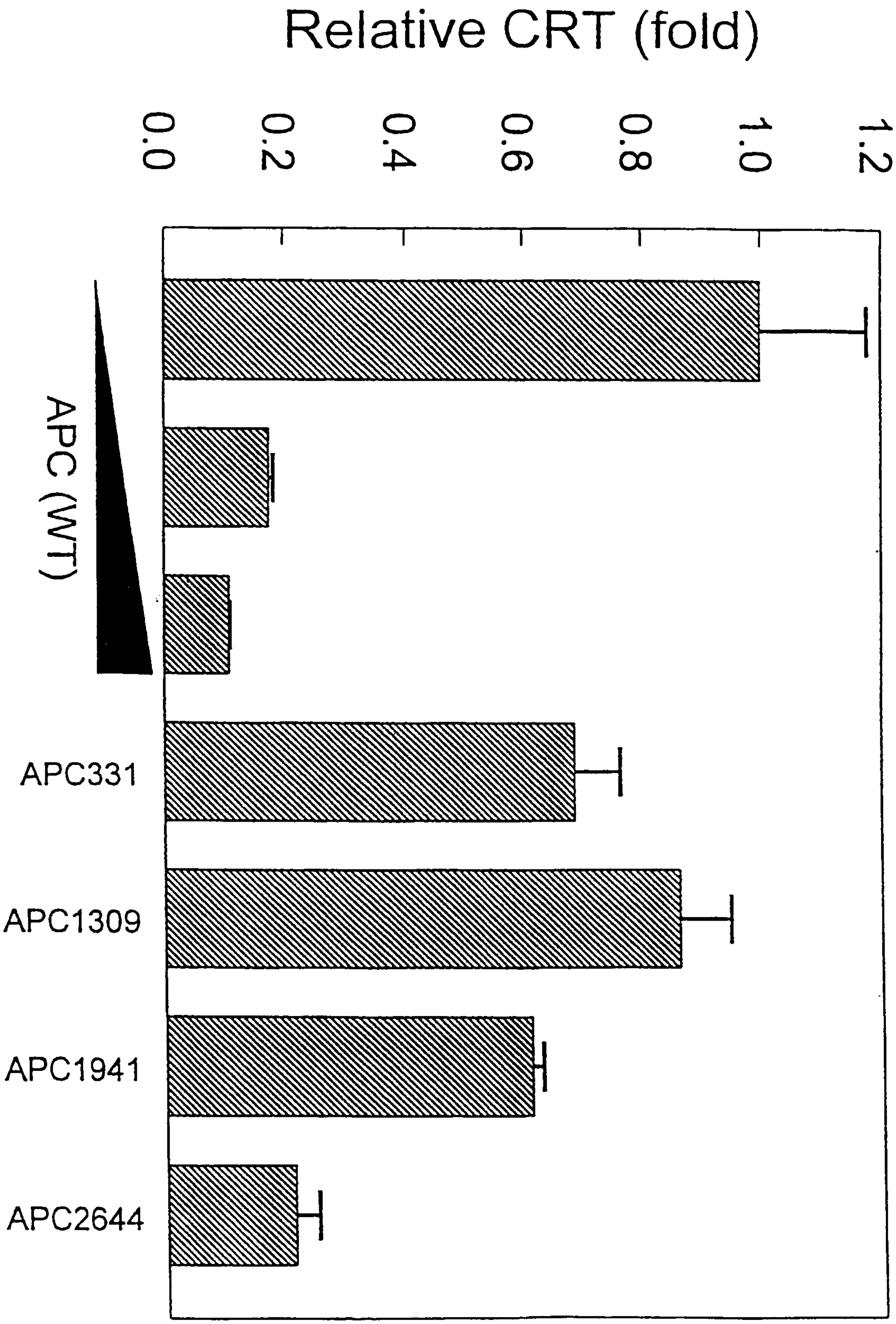

Relative CRT (fold)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
APC (WT)
APC331
APC1309
APC1941
APC2644

FIGURE 5 B

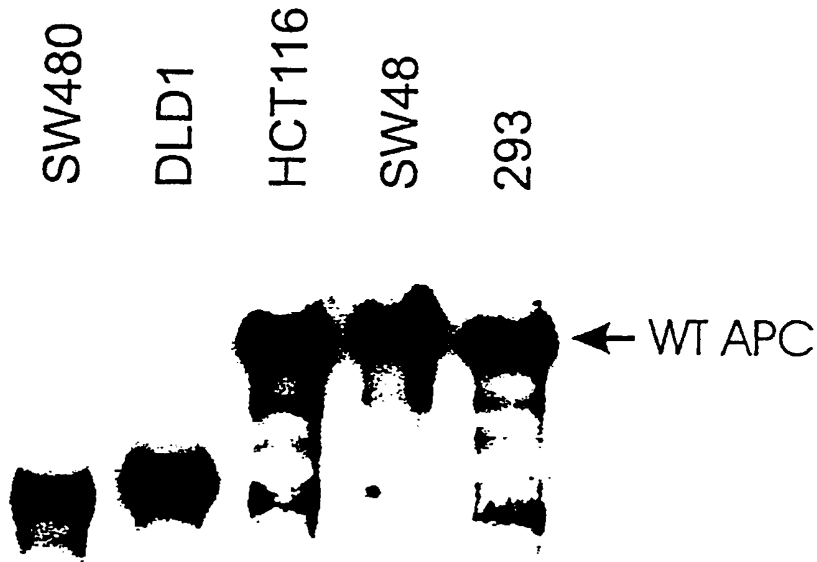


FIGURE 6A

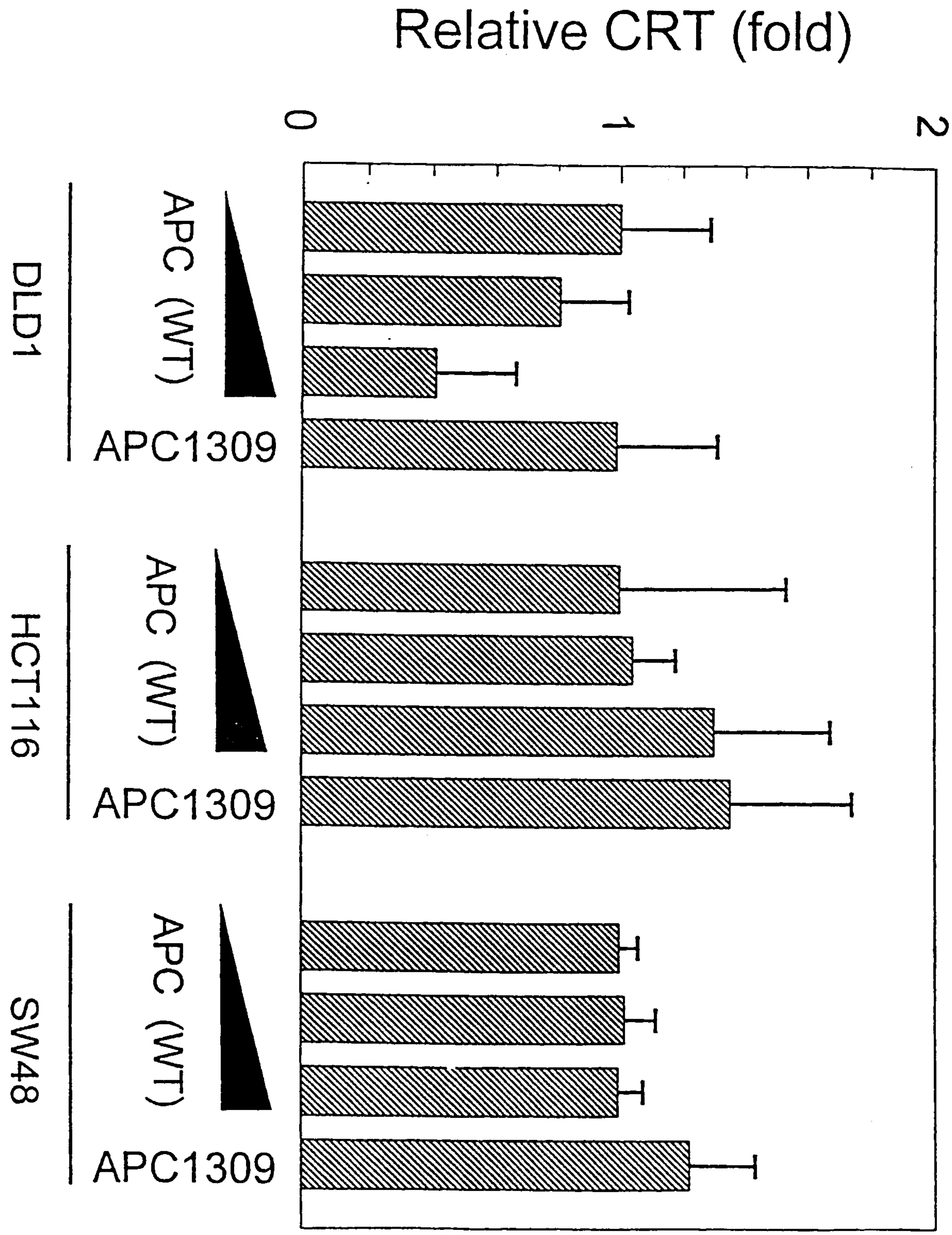

Relative CRT (fold)
0
1
2
DLD1
APC (WT)
APC1309
HCT116
APC (WT)
APC1309
SW48
APC (WT)
APC1309

FIGURE 6B

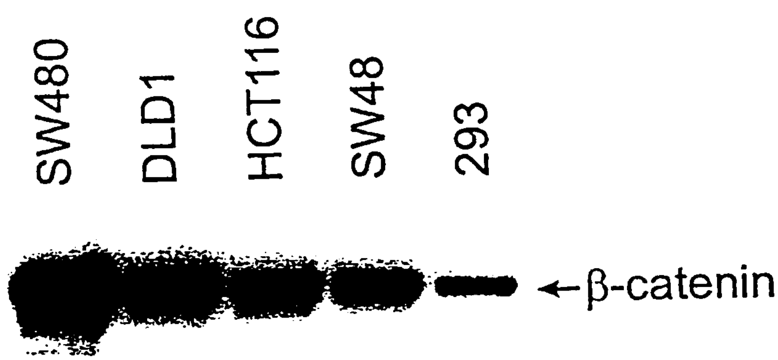


FIGURE 7A

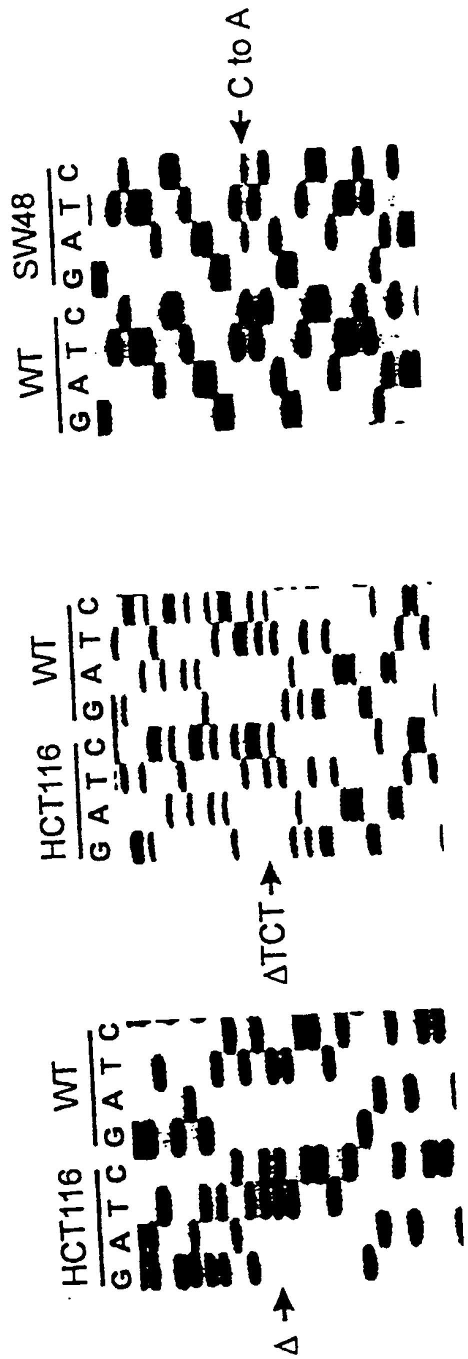

C to A
WT
G A T C
SW48
G A T C
HCT116
G A T C
WT
G A T C
ΔTCT
HCT116
G A T C
WT
G A T C
Δ

FIGURE 7 B

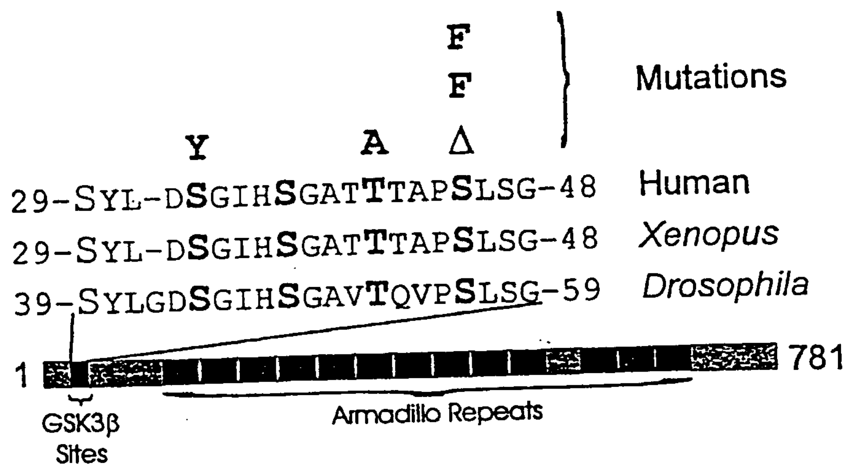

F
F
Y A Δ
Mutations
29-SYL-DSGIHSGATTTAPSLSG-48 Human
29-SYL-DSGIHSGATTTAPSLSG-48 Xenopus
39-SYLGDSGIHSGAVTQVPSLSG-59 Drosophila
1
781
GSK3β Sites
Armadillo Repeats

FIGURE 7c

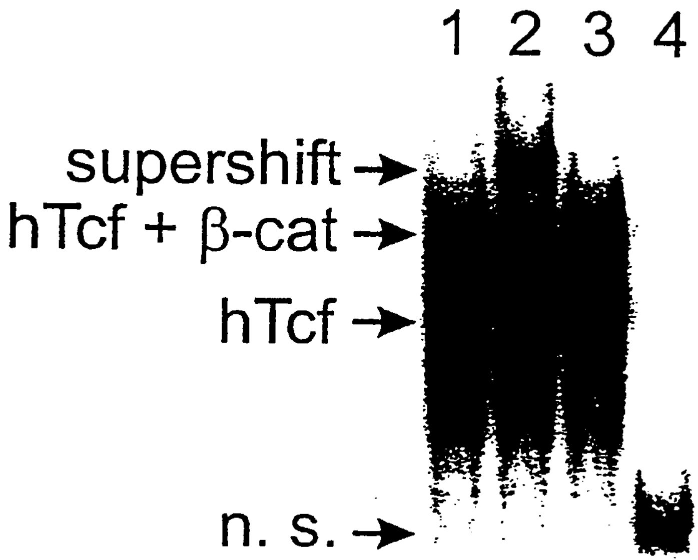
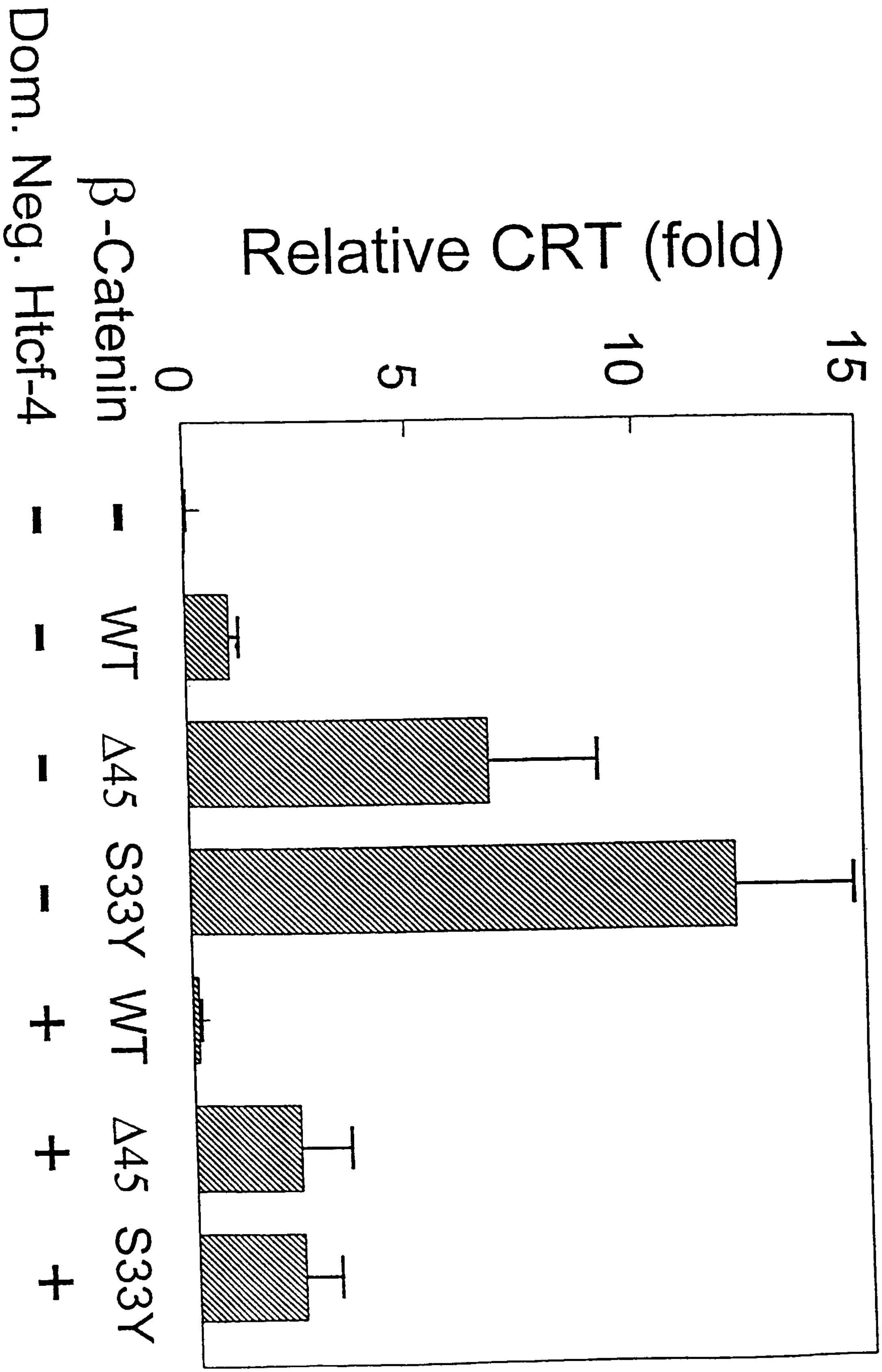


FIGURE 8A


Relative CRT (fold)
0
5
10
15
β-Catenin
Dom. Neg. Htcf-4
-
-
WT
-
Δ45
-
S33Y
-
WT
+
Δ45
+
S33Y
+